Effect of Heterozygous Mutations in CYP2D6 on Serum Concentration of Risperidone and Venlafaxine

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Thesis submitted for the degree Candidate Pharmaciae at
Department of Pharmaceutical Biosciences, School of Pharmacy,
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Kristina Fosaas

ABBREVATIONS

BBB – Blood brain barrier

C/D - ratio - Serum concentration/dose

Cl_{int} - Clearence intrinsic

CNS - Central nervous system

CYP - Cytochrome P450

DNA - Deoxyribonucleic acid

EDTA - Ethylenediaminetetraacetic acid

EM - Extensive metabolizer

HEM - Heterozygous extensive metabolizer

IR - Immediate release

PCR - Polymerase chain reaction

P-gp - P-glycoproteine

PM - Poor metabolizer

SNP - Single nucleotide polymorphism

TDM - Therapeutic drug monitoring

UM - Ultra rapid metabolizer

XR - Extended release

9-OH risperidone - 9-hydroxyrisperidone

ABSTRACT

Introduction: Both risperidone and venlafaxine are metabolized by Cytochrome P450 2D6 (CYP2D6). Genetic polymorphism in this enzyme may contribute to the significant variation in pharmacokinetics for these substances. It has previously been shown that systemic exposure of risperidone and venlafaxine is increased in patients with homozygous mutations in CYP2D6 (poor metabolizers). The possible influence of heterozygous mutations in CYP2D6 on systemic exposure is not well investigated for these drugs.

Aim: The aim of the present analysis was to investigate the importance of heterozygous mutations in CYP2D6 for serum concentrations of venlafaxine and risperidone and their main metabolites.

Method: Data were collected from a therapeutic drug monitoring database at the Department of Psychopharmacology at Diakonhjemmet Hospital. The patient samples were requested as a part of follow-ups and control of drug treatment. Steady-state serum concentrations of parent compound and metabolite as well as CYP2D6, CYP2C9 and CYP2C19 genotypes were collected for all patients. For risperidone, both tablets and long-acting injection were included in this analysis.

Result: The present analysis showed that heterozygous extensive metabolizers (n=23) had significantly higher ($p<0.01$) concentration/dose ratio of risperidone compared to CYP2D6 extensive metabolizers (n=42) after administration of risperidone tablets. Extensive metabilizers (n=32) on risperidone long-acting injection had significantly higher ratio of 9-hydroxyrisperidone and risperidone ($p<0.03$) compared to heterozygous extensive metabolizers (n=12) for 9-hydroxyrisperidone/risperidone ratio. CYP2D6 extensive metabolizers on venlafaxine (n=52) had significantly higher levels of *O*-desmethylvenlafaxine ($p<0.02$) and *O*-desmethylvenlafaxine/venlafaxine ratio ($p<0.01$) compared to CYP2D6 heterozygous extensive metabolizers (n=41). Concentration/dose ratio of *N*-desmethylvenlafaxine was significantly higher (9-fold higher) ($p<0.01$) in CYP2D6 heterozygous extensive metabolizers (n=6) compared to CYP2D6 extensive metabolizers (n=13).

Conclusion: Common for risperidone and venlafaxine is that even though the sum of the active moiety not is changed, the relative contribution of parent compund and active metabolite to the sum is altered. It is the sum of the active moiety that is determined in therapeutic drug monitoring, and patients with different CYP2D6 genotypes are therefore

treated equally in clinical practice as the sum will be the same independent of genotype. This could be a problem if there is a difference in effect and/or adverse drug reactions mediated by parent drug and metabolite.

1. INTRODUCTION

1.1 Variation in drug response

The response to given dose of a drug may vary significantly between two persons who are given the same dose of drug. Variation in drug response may be caused by variation in pharmacokinetics and/or pharmacodynamics. This variation may be genetically, physiologically, pathophysiologically or environmentally influenced (Eichelbaum et al, 2006). Genetic factors may account for as much as 95% of interindividual differences in serum concentration of drugs (Kalow et al, 1998). Examples of factors which may potentially affect systemic exposure of drugs are age, weight, organ function, interactions with other drugs, pregnancy, smoking and diet.

1.2 CYP enzyme system

An important contributor to variation in drug response is variation in drug metabolism. The cytochrome P-450 (CYP) enzyme is a group of enzymes that change substances to more hydrophilic metabolites which are more easily eliminated from the body. The role of CYP-enzymes is to detoxify foreign substances, including many drugs. The most important CYP-enzymes in the metabolism of psychotropic drugs are CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 (Dahl, M. L., 2002). CYP2D6, CYP2C9 and CYP2C19 show genetic polymorfism and for these enzymes a total lack of and/or reduced enzyme activities have been identified (Table 1.1). For CYP2D6 and CYP2C19, mutations that results in increased enzyme activity have also been detected.

The different genotypes will give different drug exposure and thereby unpredictable risk for side effects and failure of therapy. Poor metabolizers (PM) have inactivating mutations in both gene copies and therefore do not produce active enzyme. Standard dosing with a drug that is mainly metabolized by this enzyme often results in higher serum concentrations than expected according to dose, and thereby increased risk of overdose and side effects. PM should therefore be treated with lower dose than what is normal for the drug, as long as it is the drug that is pharmacologic active, and not the metabolite. Approximately 7 % of

Caucasians are CYP2D6 PM (Dahl et al, 1995). Heterozygote extensive metabolizers (HEM) have inactivating mutation in one gene copy and one gene copy that produces fully active enzyme. The result of heterozygote mutations may be an intermediate metabolic capacity, and therefore HEM may risk higher serum concentrations than what is expected in proportion to dose. Homozygote extensive metabolizers (EM) have no mutations and therefore have two gene copies that can produce fully active enzyme (wild type). Ultra rapid metabolizers (UM) have more than two gene copies and metabolize drugs by CYP2D6 faster than EM. The overall frequency of the amplified CYP2D6-allele is about 1% in Caucasians (Dahl et al, 1995). Risk for failure of drug therapy is high when treating UM with standard doses of drugs that are metabolized by CYP2D6, if it is the drug, and not the metabolite that is active. The clinical importance of mutations is dependent on the relative importance of the mutated CYP enzyme for the elimination of the drug as well as the capacity in alternative eliminating pathways.

1.3 Therapeutic drug monitoring (TDM)

Serum concentration measurement is an important tool in order to minimize adverse drug reactions and optimize therapeutic response. Serum concentration measurement is important in psychiatry because many psychotrophic drugs are metabolized by CYP2D6 and in addition many psychiatric patients have poor compliance. The use of serum concentration measurement is especially indicated for drugs with a small therapeutic range, for drugs with well documented relation between concentration and effect and/or side effects, and for drugs with a wide pharmacokinetic variability (Rudberg et al, 2005). Especially some groups of patients can benefit from TDM, for instance the elderly, children, breast-feeding women and pregnant women, patients using multiple drugs, patients with poor compliance, and patients with decreased organ function (Rudberg et al, 2005).

1.4 CYP-genotyping

Genotyping of CYP-enzymes is useful when the patient is using a drug which has serum concentration that in a high degree is determined by the genotype of a CYP enzyme. CYP-genotyping would be an advantage before starting with some drugs, for instance warfarin that is metabolized by CYP2C9, as serum concentration related side-effects of warfarin might have a fatal outcome (Wadelius and Pirmohamed, 2006). Other reasons given for CYP-

genotyping are failure of therapy and/or side-effects in patients that already have been medicated with the drugs (Rudberg et al, 2005). If genotyping was performed before the patients were medicated, precautions, like for instance adjusted drug dosage or a change of medicine, could have been taken.

Table 1.1 An overview of the most mutations in CYP2D6, CYP2C9 and CYP2C19.

Enzyme	Mutation	Enzyme activity	Allele frequency in Caucasians
CYP2C9	**2*: Amino acid change R144C, 430C>T	Reduced	11 % [1]
	**3*: Amino acid change I359L, 1075A>C	Reduced	9 % [1]
	**5*: Amino acid change D360E, 1080C>G	Reduced	Rare [2]
CYP2C19	**2*: Splice defect 681G>A	None	15 % [3]
	**3*:Stopcodon 636G>A	None	8,3 % [3]
	**4*:GTG initial codon 1A>G	None	Rare [4]
	**5*: Amino acid change R433W, 1297C>T	None	< 0,9 %[4]
	**17*: Amino acid change 806C>T, 3402C>T	Increased	18%[5]
CYP2D6	**2*: Duplication of CYP2D6 gene	Increased	1-2 % [6,7]
	**3*: Readingframe change 2549A>del	None	2,7 % [8]
	**4*: Splice defect 1846G>A	None	21,5-28,6 % [8]
	**5*: Deletion of CYP2D6 gene	None	3 % [8]
	**6*: Readingframe change 1707T>del	None	1 % [7]
	**7*: Amino acid change H324P, 2935A>C	None	Rare [8]
	**8*: Stop codon 1758G>T	None	Rare [8]
	**9*: Base-pair deletion 2613_2615delAGA	Reduced	1-2%[7]
	**10*: Amino acid change 100C>T	Reduced	<2%[7]
	**17*:Amino acid change 1111C>T, 2938C>T , 4268G>C	Reduced	Rare[7]

[1] Scordo et al, 2001, [2]Yasar et al, 2002, [3]Jose et al, 2004, [4]Ibenau et al, 1998, [5]Sim et al, 2005 [6]Dahl et al,1995, [7]Bradford, 2002, [8]Zackrisson et al, 2003

1.5 Risperidone

Risperidone is the active substance in the atypical antipsychotic drug Risperdal® which inhibits dopaminerg and serotonerg transmission in the central nervous system (CNS). (Leysen et al, 1988). Risperidone is administered as tablets, long-lasting injection (Risperdal Consta®), mixture and melting tablets. Risperidone tablets are dosed 1-2 times daily. Risperidone injection is an option for the maintenance therapy of schizophrenia and with repeated injections every two weeks, steady state levels are usually reached after three injections (Knox et al, 2004). Patients receiving risperidone long-acting injection has

significant lower serum concentrations of risperidone and its main metabolite 9-hydroxyrisperidone than patients taking equivalent doses oral risperidone. Patients receiving risperidone long-lasting injection also have lower 9-hydroxyrisperidone/risperidone ratio than patients taking oral risperidone (Nesvåg et al, 2006).

Risperidone is metabolized mainly by CYP2D6 to the pharmacologically active 9-hydroxyrisperidone, but also CYP3A enzymes are found to be involved in the metabolism of risperidone to 9-hydroxyrisperidone (Fang et al, 1999) (Figure 1.1). An *in vitro* study has shown that intrinsic clearence (Cl_{int}) for risperidone was about 20 times higher for CYP2D6 than for CYP3A4 (Fang et al, 1999). Terminal half life for risperidone is about 6-7 hours, and for 9-hydroxyrisperidone about 24 hours (Riedel et al, 2004). The sum of risperidone and 9-hydroxyrisperidone form the active moiety *in vivo*. In TDM, the two compounds are summarized, following Consensus Guidelines (Baumann et al, 2004).

Genetic polymorphism has been shown to be an important contribution to pharmacokinetic variability of risperidone (Aravagiri et al, 2003). CYP2D6 PM has significantly higher serum concentration of risperidone compared to CYP2D6 EM after administration of risperidone tablets (Mihara et al, 2003, Scordo et al, 1999), as well as an increased risk of side-effects (de Leon et al, 2005). In addition, Scordo and co-workers showed that for patients on risperidone tablets, CYP2D6 HEM had significantly higher ratios of risperidone/9-hydroxyrisperidone compared to CYP2D6 EM, while there were no significant differences between the two groups for the C/D ratio of the risperidone, C/D ratio of 9-hydroxyrisperidone or C/D ratio of the active moiety (Scordo et al, 1999). The same was also seen in another study (Riedel et al, 2005). For risperidone long acting injection, there are no investigations on the significance of CYP2D6 genotype for serum concentration of risperidone and 9-hydroxyrisperidone.

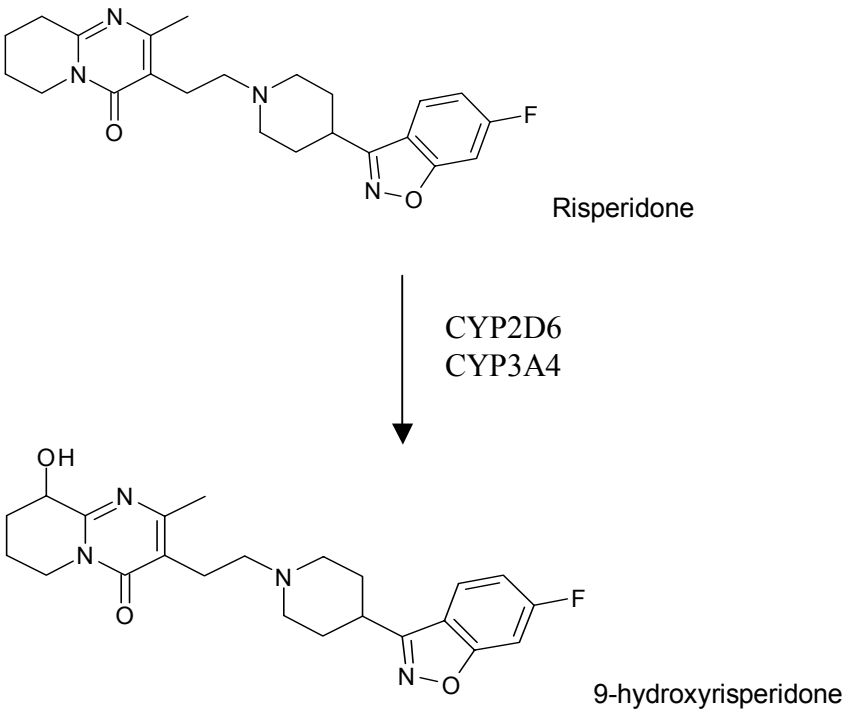

Figure 1.1 Metabolism of risperidone by CYP2D6 and CYP3A4.

1.6 Venlafaxine

Venlafaxine, the active substance in the antidepressant Efexor®, inhibits reuptake of serotonin and noradrenalin in CNS (Muth et al, 1986). Some inhibition of dopamine reuptake has been seen. Venlafaxine is administered as either immediate release (IR) or extended release (XR) tablets. XR formulation provides prolonged absorption of active drug in comparison with IR formulation, but total absorption of venlafaxine is the same (Entsuah et al, 1997). It has been demonstrated that XR gives less of the side-effects nausea and dizziness than IR (Entsuah et al, 1997). In addition, the XR formulation gives better patient compliance because it is taken once daily, in contrast to the IR formulation which is administered 2-3 times daily. Therefore, mainly XR tablets are in use today.

Venlafaxine is metabolized mainly through CYP2D6 to the pharmacologically active metabolite *O*-desmetylvenlafaxine and to a minor degree to the pharmacologically inactive *N*-desmethylvenlafaxine through CYP3A4, CYP2C9 and CYP2C19 (Figure 1.2) (Otton et al, 1996). In low venlafaxine concentrations *in vitro,* the contribution to the formation of *O*-desmetylvenlafaxine by CYP2D6, CYP2C19 and CYP2C9 were estimated to be 89%, 10% and 1%, respectively, based on estimated Cl_{int} (Fogelman et al, 1999). For *N*-

desmethylvenlafaxine, the contribution by CYP3A4, CYP2C19 and CYP2C9 are 36%, 33% and 31%, respectively (Fogelman et al, 1999). *O*-desmetylvenlafaxine and *N*-desmethylvenlafaxine have been suggested to be metabolized further to the metabolite *N, O*-didesmethylvenlafaxine (Muth et al, 1991) (Figure 1.2). *O*-desmethylvenlafaxine displays similar serotonin reuptake inhibition activity and two-fold higher noradrenalin reuptake inhibition than venlafaxine; while *N*-desmethylvenlafaxine and *N, O*-didesmetylvenlafaxine are pharmacologically inactive (Muth et al, 1991). In TDM, venlafaxine and O-desmethylvenlafaxine are summarized, following Consensus Guidelines (Baumann et al, 2004). The terminal half – life of venlafaxine and *O*-desmetylvenlafaxine are 5 hours and 11 hours, respectively (Veefkind et al, 2000).

Genetic polymorphism has been shown to be an important contribution to pharmacokinetic variability of venlafaxine. CYP2D6 PM have been shown to have an increased venlafaxine serum concentration in comparison to CYP2D6 EM (Veefkind et al, 2000, Fukuda et al, 2000), while the sum of concentration venlafaxine plus *O*-desmetylvenlafaxine has been shown not to significantly differ between the different genotypes (Shams et al, 2006). The effect of heterozygote mutations in CYP2D6 on serum concentration of venlafaxine and metabolites is not known because the number of CYP2D6 HEM in these studies has been too low to study these patients separately (Veefkind et al, 2000, Fukuda et al, 2000).

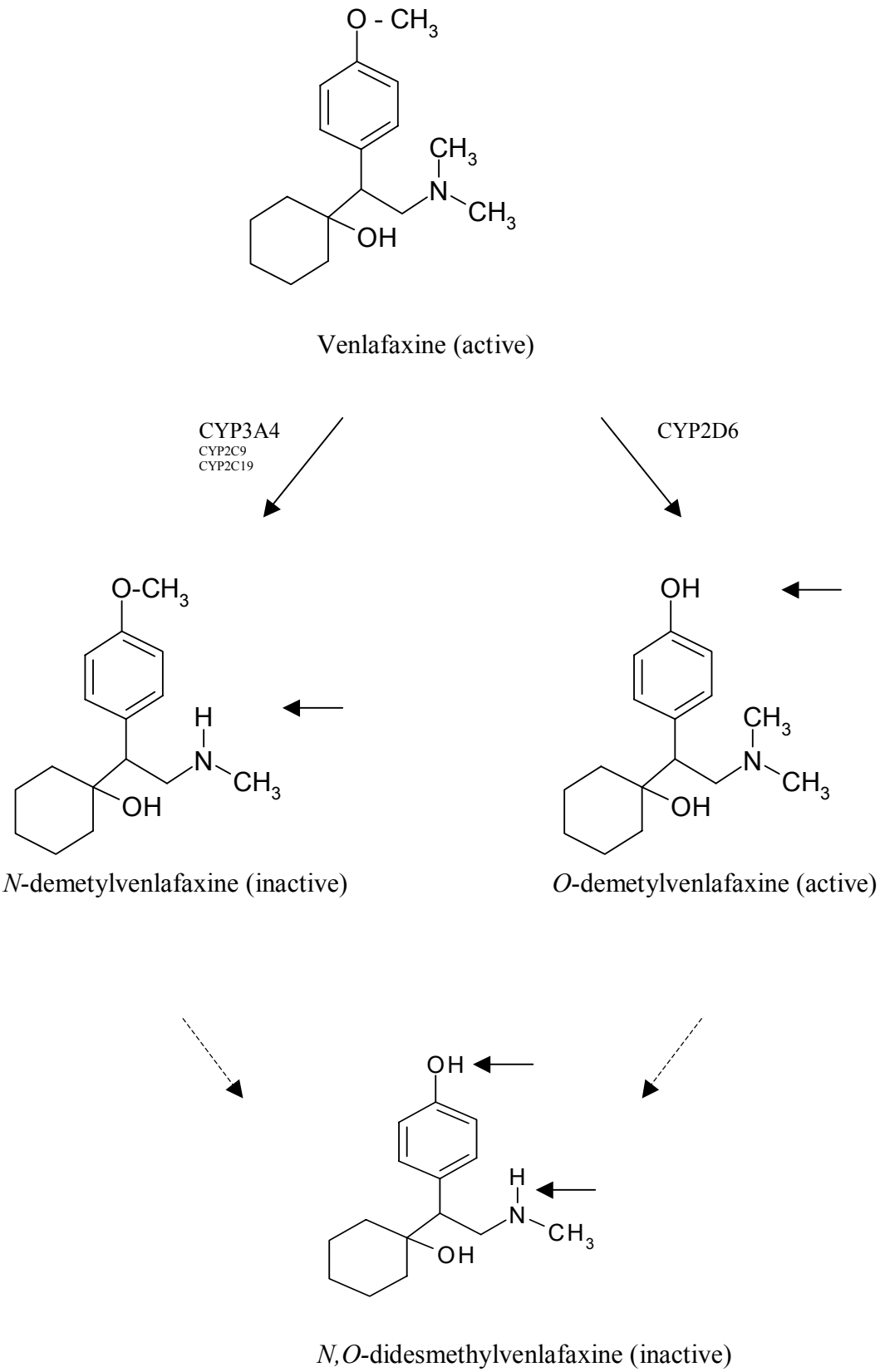

Figure 1.2 Metabolism of venlafaxine.

1.7 Aims

The aim of the present study was to investigate the importance of heterozygous mutations in CYP2D6 for serum concentrations of venlafaxine and risperidone (tablets and long-acting injection) and their main metabolites.

In addition, analyses were done for:

• The importance of heterozygous mutations in CYP2D6 for steady state serum concentrations of *N*-desmethylvenlafaxine.

• The possible influence of mutations in CYP2C9 and CYP2C19 on the steady state serum concentration of venlafaxine.

2. METHODS

2.1 Patient population and study design

Data were collected from a TDM database at the department of Psychopharmacology at Diakonhjemmet Hospital. The patient samples were taken as a part of standard clinical follow-ups and routine control of drug treatment. For all the patients included in the analysis, CYP genotyping and serum analysis of risperidone and venlafaxine were performed in the period from January 2001 to August 2006. In order to be included in the study, the samples for serum analysis had to be drawn at steady state conditions. This implies that the patient must have been on stable dose of risperidone or venlafaxine, at least three days prior to blood sampling. For risperidone injection the requirement was at least three injections prior to sampling. In addition, samples must have been drawn 12-24 hours after previous oral dose, or 13-15 days after previous injection of risperidone. Patients with a high probability of poor compliance were excluded from the study. Because of the possible drug-drug interactions samples were excluded from analysis when information on concomitant use of the CYP3A4 inducing drug carbamazepine (Tegretol®) and the CYP2D6 inhibiting drug fluoxetine (Fontex®) was given on the request from. When more than one serum sample had been performed for the same patient, the sample nearest in time-relationship to the CYP-genotyping was selected. Sex, age, dose and time between last drug intake and sample withdrawal were registered. For patients with samples on more than one dosage that fulfilled inclusion criteria, the mean serum concentration/dose ratio was calculated. The study was approved by the regional ethical committee.

2.2 Sample analysis

2.2.1 Analysis of risperidone

The development of the method has been performed at Psychopharmacologic Department at Diakonhjemmet Hospital and the method is used for the daily routine analysis. Briefly, purified samples (protein-precipitated with acetonitril) were injected on a Waters 2795 Liquid Chromatography and Micromass Quattro micro tandem detector (both from Milford, MA, USA). Risperidone and 9-hydroxyrisperidone were separated on a C18 analytical column protected by an ACE 3AQ guard column (both ACE 3AQ Advanced Chromatography

Technologies, Aberdeen, UK) by gradient elution (10%-80% acetonitril in 10 mmol/l ammoniumacetate buffer; pH=4.5). The retention times were 4.7 minutes (risperidone), 4.3 minutes (9-hydroxyrisperidone) and 6.5 minutes (promazine, internal standard). Detection was performed with atmospheric pressure ionization after multiple reactions monitoring at transitions 411.2 → 191.1 for risperidone, 427.2 → 207.1 for 9-hydroxyrisperidone and 285.2→212.1 for promazine. LOQs for risperidone and 9-hydroxyrisperidone were 1 nM.

2.2.2 Analysis of venlafaxine

The development of the method has been performed at Psychopharmacologic Department at Diakonhjemmet Hospital and the method is used for the daily routine analysis. Briefly, purified samples (protein-precipitated with acetonitril) were injected on a Waters 2795 Liquid Chromatography and Micromass Quattro micro tandem detector (both from Milford, MA, USA). The analytes were separated on a C18 analytical column (ACE 3AQ Advanced Chromatography Technologies, Aberdeen, UK) protected by a Sunfire C18 Guard column (Waters, Ireland) by gradient elution (24%-52% acetonitril in 10 mmol/l ammoniumacetate buffer; pH=4.5). The retention times were 2.3 minutes (*O*-desmethylvenlafaxine), 3.9 minutes (*N*-desmethylvenlafaxine), 4.2 minutes (venlafaxine) and 6 minutes (protriptyline, internal standard). Detection was performed with atmospheric pressure ionization after multiple reactions monitoring at transitions 278.15 → 260.15 for venlafaxine and 264.15 → 246.15 *O*-desmethylvenlafaxine and *N*-desmethylvenlafaxine, and 264 →233.2 protriptyline. LOQs for venlafaxine, *O*-desmethylvenlafaxine and *N*-desmethylvenlafaxine were 10 nM.

2.2.3 CYP-genotyping

The CYP-genotyping is used in the daily routine analysis at Psychopharmacologic Department at Diakonhjemmet Hospital. Blood samples were collected in tubes containing EDTA as anticoagulant. Genomic DNA was isolated from leucocytes by E.Z.N.A® Blood DNA Kits II (Omega Bio-tek, Doraville, GA 30362, USA). All the polymerace chain reaction (PCR) amplification was carried out using Applied Biosystem 7500 Real-Time PCR instrument with Sequence Detection Software (SDS), Version 1.3 (Applied Biosystem, CA 94404, USA). *CYP2D6*2* (duplication) and *CYP2D6*5* (deletion) mutations were determined by PCR reaction using a specific set of allele amplification primers (Schaeffler et al, 2003).

C*YP2C9*2, *3, *5, CYP2C19*2, *3, *4, *5, CYP2D6*3, *4, *6, *7* and **8* mutations were determined by use of a long-range PCR amplifying the whole CYP2D6 gene, followed by multiplex allele specific PCR. The preamplication was diluted with water and used as template for two separate PCR reactions for the multiplex allele-specific PCR. The PCR reaction mix for each reaction was containing genomic DNA, specific primers, nucleoside triphosphates (dATP, dGTP, dCTP and dTTP), $MgCl_2$ or $Mg(OAc)_2$, buffer and ddH_2O. AmpliTaq Gold DNA Polymerase was used in the *CYP2C9, CYP2C19* and in the multiplex PCR. High fidelity DNA Polymerase was used in the *CYP2D6*2* and **5* reactions, and the rTth DNA polymerase was used in the long-range PCR. The samples were separated by electrophoresis, and the fragments were compared with albumin as a molecular weight marker, an internal reference gene which was coamplified simultaneously in a single-tube biplex assay. Single nucleotide polymorphism (SNP) analysis positive controls representative of each genotype and negative or no template controls were included in each essay. For analysis of *CYP2D6* copynumber, controls are patient samples with known genotype – normal control, duplication control and deletion control.

2.3 Statistical methods

All the statistical analysis was performed with SPSS® Software (SPSS Inc., Chicago, USA). Mann-Whitney test was used for comparing dose-adjusted serum concentrations of parent drugs, metabolites and metabolite/parent drug ratios between the different genotype groupings. $p<0.05$ was considered statistical significant.

3. RESULTS

3.1 Risperidone tablets

A total number of 68 patients fulfilled the inclusion criteria and were included in the analysis. Among these, 42 patients carried two functional *CYP2D6* alleles and 23 patients carried one functional and one defect allele. The genotypes in this latter group were *1/*3 (n=1), *1/*4 (n= 21) and *1/*5 (n=1). Among the rest, two patients carried two defect alleles, *3/*5* and 4/*6, and one patient carried more than two functional alleles.

Table 3.1 Demographic data of patients treated with risperidone tablets.

CYP2D6	EM	HEM	PM	UM
Total N (female/male)	42 (23/19)	23 (11/12)	2 (1/1)	1 (1/0)
Age, years[1]	37 (15-80)	34 (22-57)	37 (25/48)	80
Dose, mg/day[1]	4 (1-6)	4 (2-7)	3 (2-4)	3
Sample withdrawal, hours[1]	13 (11-24)	14 (11-22)	18 (13-24)	13

[1]Data are presented as median (range).

There were no significant differences in demographic data between the EM and HEM groups (Table 3.1). Due to a low number of patients in the CYP2D6 PM and CYP2D6 UM groups, statistical testing was not considered meaningful for these groups.

Table 3.2 Steady-state serum concentration-to-dose (C/D) ratios of risperidone, 9-hydroxyrisperidone and active moiety (sum of risperidone and 9-hydroxyrisperidone) and the ratio of 9-hydroxyrisperidone/risperidone in different CYP2D6 genotypes after administration of risperidone tablets.

CYP2D6	EM	HEM	PM	UM
Total N	42	23	2	1
C/D risperidone, nM per mg[1]	0.9 (0-31.3)	7.9 (0-49.8)*	21.9 (20.3-23.5)	0.8
C/D 9-hydroxyrisperidone, nM per mg[1]	10.9 (2.8-31)	9.3 (2.7-29)	6.5 (6.5-6.5)	23.9
C/D active moiety, nM per mg[1]	13.6 (2.8-45)	16.3 (2.7-60.8)	28.4 (26.8-30)	24.7
9-hydroxyrisperidone/risperidone[1]	7.2 (0-37.5)	1.1 (0-38.5)	0.3 (0.3-0.3)	31.9

[1]Data are presented as median (range).
* Significant difference ($p<0.01$) CYP2D6 HEM versus CYP2D6 EM.

The steady-state serum concentrations of risperidone ranged from <1 to 199 nM and those of 9-hydroxyrisperidone from 6 to 113 nM (Table 3.2). Eight patients had steady state serum

concentrations under LOQ for risperidone. As the daily dose of risperidone varied between 1 and 7 mg, the measured concentrations of risperidone and 9-hydroxyrisperidone were corrected for the dose (concentration/dose; nM per mg). The C/D ratio of risperidone was eight-fold higher in the CYP2D6 HEM group than in the CYP2D6 EM group ($p<0.01$) (Table 3.2, figure 3.1 a). The corresponding values for 9-hydroxyrisperidone did not show statistical difference between the groups; however median in the CYP2D6 HEM group tended to be lower in the CYP2D6 EM group (Figure 3.1 b). There were no significant differences in C/D ratio of the active moiety (sum of risperidone plus 9-hydroxyrisperidone) between the CYP2D6 EM and CYP2D6 HEM groups (Figure 3.1 c). No significant differences were found in 9-hydroxyrisperidone/risperidone ratio between the CYP2D6 EM and CYP2D6 HEM groups, however median in the HEM group appeared to be lower than the CYP2D6 EM group (Table 3.2, figure 3.1 d).

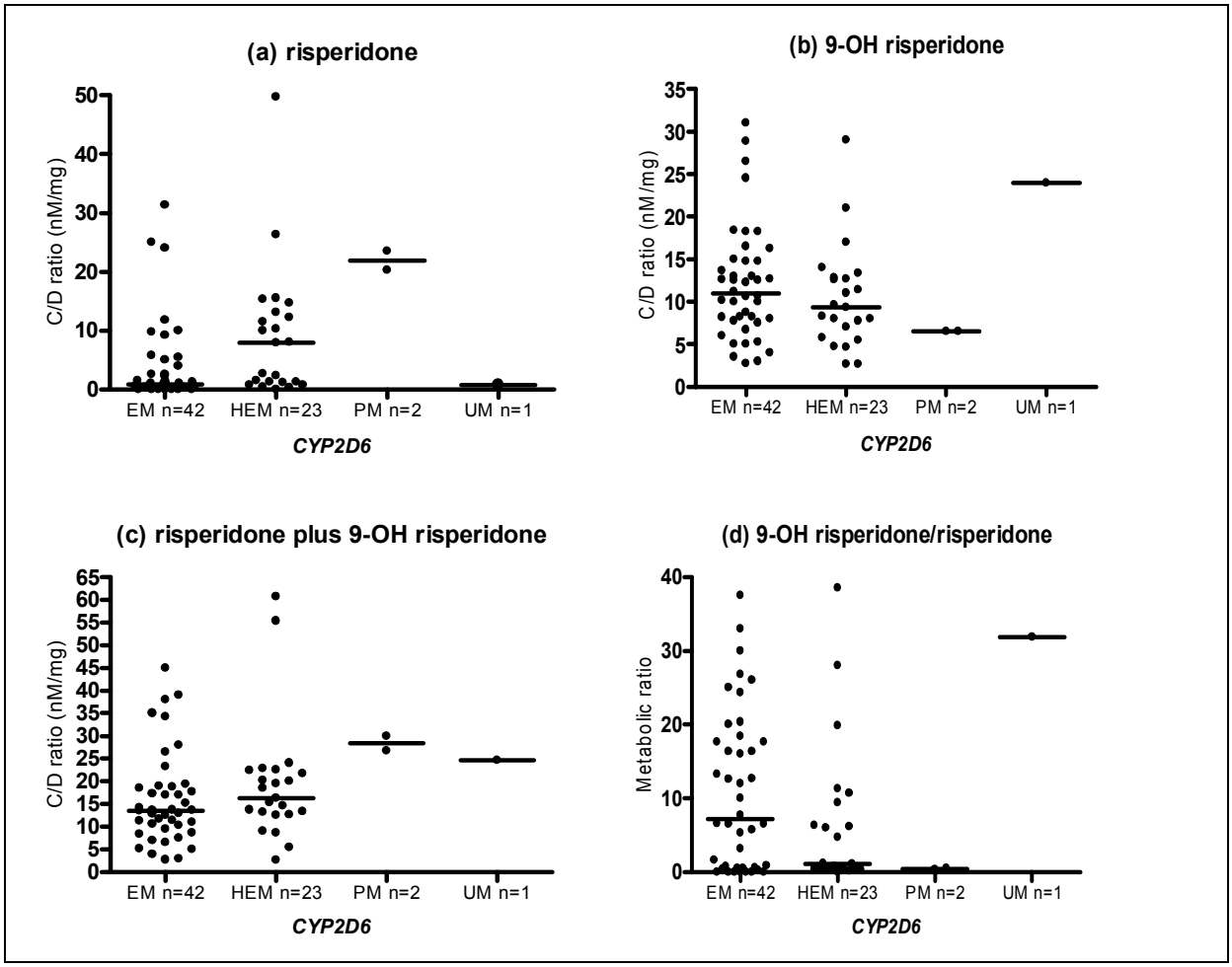

Figure 3.1 Relationship between CYP2D6 genotype and C/D ratio of (a) risperidone, (b) 9-hydroxyrisperidone, (c) risperidone plus 9-hydroxyrisperidone and (d) metabolic ratio (9-hydroxyrisperidone/risperidone) after administration of risperidone tablets.

3.2 Risperidone long-acting injection

A total number of 50 patients were included in the study. Among these, 32 patients carried two functional *CYP2D6* alleles and 12 patients carried one functional allele and one defect allele, *1/*4. Among the rest, four patients carried two defect *CYP2D6* alleles, *3/*4 (n=2) and *4/*5 (n=2) and two patients carried more than two functional *CYP2D6* alleles.

Table 3.3 Demographic data of patients treated with risperidone long-acting injection.

CYP2D6	EM	HEM	PM	UM
Total N (female/male)	32 (12/20)	12 (4/8)	4 (1/3)	2 (2/0)
Age, years[1]	38 (17-63)	29 (20-62)	34 (27/53)	65 (48-81)
Dose, mg/two weeks[1]	44 (25-75)	38 (25-50)	53 (50-75)	38 (25-50)
Sample withdrawal, days[1]	14 (13-16)	14 (13-15)	14 (13-14)	13 (13-13)

[1]Data are presented as median (range).

There were no significant differences in distribution of sex, age and time-interval between last injection and sample withdrawal between CYP2D6 EM and CYP2D6 HEM groups. However, the CYP2D6 EM group received significantly higher doses of risperidone than the CYP2D6 HEM group ($p=0.03$). Due to a low number of patients in the CYP2D6 PM and CYP2D6 UM groups, statistical testing was not considered meaningful for these groups.

Table 3.4 Steady-state serum concentration-to-dose (C/D) ratios of risperidone, 9-hydroxyrisperidone and active moiety (sum of risperidone and 9-hydroxyrisperidone) and ratio of 9-hydroxyrisperidone/risperidone in different CYP2D6 genotypes after administration of risperidone long-acting injection.

CYP2D6	EM	HEM	PM	UM
Total N	32	12	4	2
C/D risperidone, nM per mg[1,2]	0.3 (0.1-1.1)	0.3 (0.1-2.7)	0.8 (0.4-1.2)	0.3 (0.2-0.4)
C/D 9-hydroxyrisperidone, nM per mg[1,2]	0.9 (0.2-1.7)	0.7 (0.1-1.4)	0.1 (0.1-0.2)	1.2 (0.2-2.2)
C/D Sum active moiety, nM per mg[1,2]	1.2 (0.5-2.2)	1.2 (0.4-3.4)	1 (0.5-1.3)	1.5 (0.5-5.4)
9-hydroxyrisperidone /risperidone[1]	3.2 (0.2-5.5)	1.4 (0.3-4.1)*	0.2 (0-0.3)	3.2 (0.5-5.4)

[1]Data are presented as median (range).
[2]mg/two weeks
* Significant difference ($p<0.03$) CYP2D6 HEM versus CYP2D6 EM genotype.

The steady-state serum concentrations of risperidone ranged from 5 nM to 89 nM and those of 9-hydroxyrisperidone from 11 nM to 86 nM (Table 3.4). As the risperidone injections are administered every two weeks, the measured concentrations of risperidone were corrected for the dose/two weeks in the same manner as risperidone tablets were corrected for dose/day.

The 9-hydroxyrisperidone/risperidone ratio was a significantly higher in the CYP2D6 EM group compared with the CYP2D6 HEM group ($p<0.03$) (Figure 3.2 d). The C/D ratio of risperidone was not significantly different between the CYP2D6 genotypes (Figure 3.2 a). No

significant differences in the C/D ratios of 9-hydroxyrisperidone were found, however, the median in the CYP2D6 EM group seemed higher than median in the CYP2D6 HEM group (Figure 3.2 b). There was no significant difference in C/D ratio of active moiety between the CYP2D6 EM and CYP2D6 HEM (Figure 3.2 c).

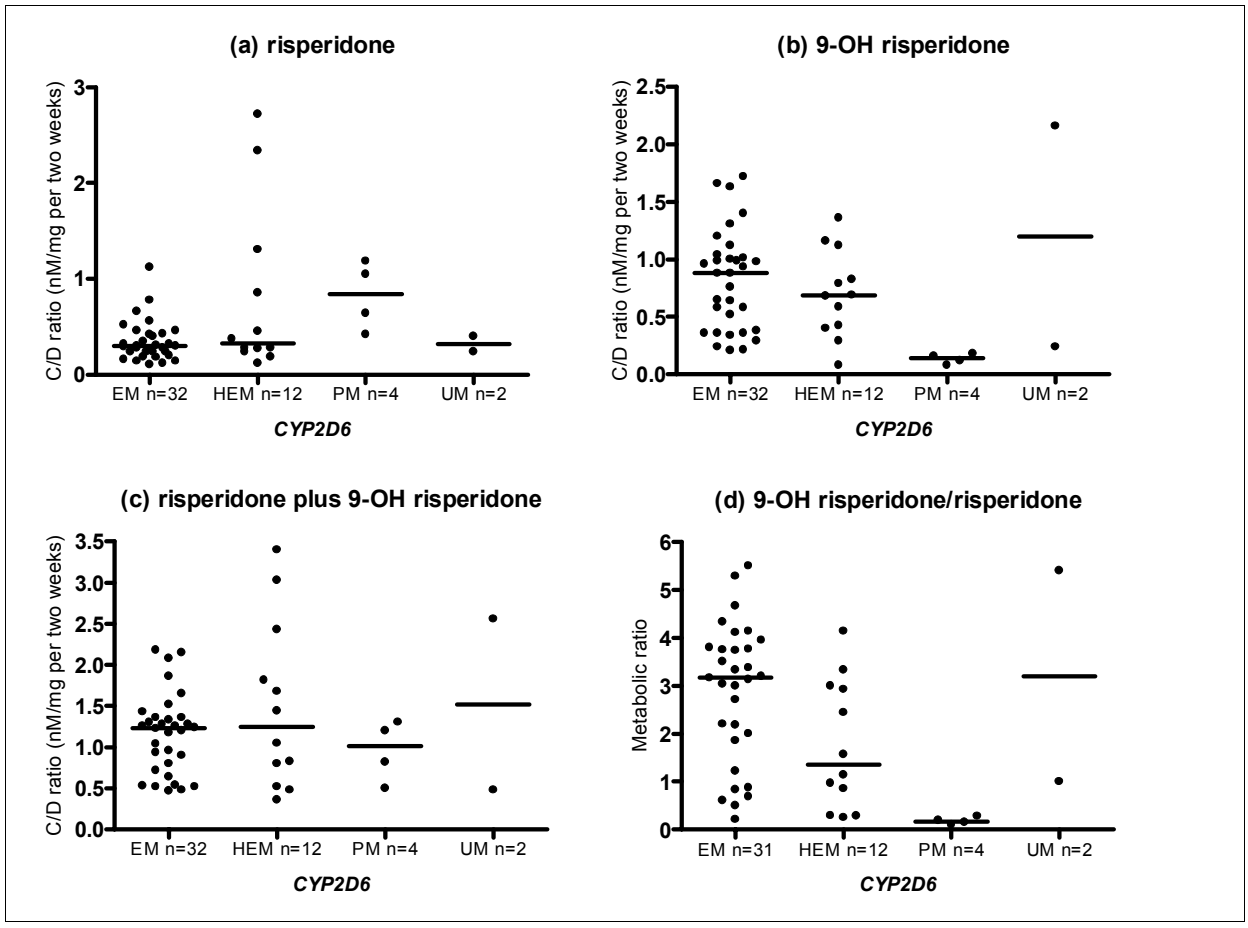

Figure 3.2 Relationship between CYP2D6 genotype and C/D ratio of (a) risperidone, (b) 9-hydroxyrisperidone, (c) risperidone plus 9-hydroxyrisperidone and (d) metabolic ratio (9-hydroxyrisperidone /risperidone) after administration of risperidone long-acting injection.

3.4 Venlafaxine

A total number of 98 patients were included in the analysis. Among these, 52 patients carried two functional *CYP2D6* alleles and 41 patients carried one functional and one defect allele. The genotypes in this latter group were *1/*3 (n=1), *1/*4 (n= 34), *1/*5 (n=5) and *1/*6 (n=1). Among the rest, one patient carried two defect alleles, *4/*5 and four patient carried

more than two functional alleles. Furthermore, for 19 of the 98 patients the N-desmethylvenlafaxine concentration was determined. Among these, 13 patients carried two functional *CYP2D6* alleles, six patients carried one functional and one defect allele, *1/*4.

Table 3.5 Demographic data of patients treated with venlafaxine tablets.

CYP2D6	EM	HEM	PM	UM
Total N (female/male)	52 (31/21)	41 (26/15)	1 (1/0)	4 (3/1)
Age, years[1]	39 (13-80)	39 (17-69)	45	41 (32-44)
Dose, mg/day[1]	150 (37.5-375)	150 (37.5-375)	300	113 (75-300)
Sample withdrawal, hours[1]	18 (11-25)	16 (11-24)	20	12 (12-13)
Number of *CYP2C9* EM	30	28	0	2
Number of *CYP2C9* HEM	17	6	1	1
Number of *CYP2C9* PM	2	0	0	1
Number of unknown *CYP2C9*	3	7	0	1
Number of *CYP2C19* EM	32	21	1	3
Number of *CYP2C19* HEM	18	13	0	0
Number of unknown *CYP2C19*	3	7	0	1

[1]Data are presented as median (range).

There were no significant differences in demographic data between the CYP2D6 EM and HEM groups (Table 3.5). Due to a low number of patients in the CYP2D6 PM and CYP2D6 UM groups, statistical testing was not considered meaningful for these groups.

Table 3.6 Steady-state serum concentration–to-dose (C/D) ratios of venlafaxine, *O*-desmethylvenlafaxine, *N*-desmethylvenlafaxine and active moiety (sum of venlafaxine and *O*-desmethylvenlafaxine) and ratios of *O*-desmethylvenlafaxine/venlafaxine and *N*-desmethylvenlafaxine/venlafaxine in different CYP2D6 genotypes.

CYP2D6	EM	HEM	PM	UM
Total N	52	41	1	4
C/D venlafaxine, nM per mg[1]	1.5 (0.2-9.1)	1.9 (0.3-20.0)	2.2	3.6 (0.4-6.2)
C/D *O*-desmethylvenlafaxine, nM per mg[1]	3.9 (1.0-10.7)	3.1 (0.1-7.7)*	3.2	5.3 (2.3-8.9)
C/D Sum active moiety, nM per mg[1]	5.3 (1.2-16.9)	5.2 (1.5-20.1)	5.4	9.4 (5.8-11.1)
O-desmethylvenlafaxine/venlafaxine[1]	3.2 (0.3-15.6)	1.6 (0.1-8.5)**	1.5	3.6 (0.4-13.5)
C/D *N*-desmethylvenlafaxine, nM per mg[1,2]	0.2 (0-1.8)	1.9 (0.2-3.7)***		
N-desmethylvenlafaxine/venlafaxine[1,2]	0.2 (0-0.7)	0.5 (0.2-1.5)		

[1]Data are presented as median (range).
[2]The serum concentration of *N*-desmethylvenlafaxine was determined for 19 of the 98 patients.
* Significant difference ($p<0.02$) *CYP2D6* EM versus CYP2D6 HEM genotype.
** Significant difference ($p<0.01$) *CYP2D6* EM versus CYP2D6 HEM genotype.
*** Significant difference ($p<0.01$) *CYP2D6* EM versus CYP2D6 HEM genotype.

The steady-state serum concentrations of venlafaxine ranged from 21 nM to 2996 nM, those of *O*-desmethylvenlafaxine from 22 nM to 2040 nM and those of N-desmethylvenlafaxine from <10 nM to 998 nM. Three patients had steady state serum concentrations under LOQ for N-desmethylvenlafaxine. Due to a wide range of daily doses (37.5 mg to 375 mg), measured concentrations were corrected for the doses (Table 3.6).

The C/D ratio of *O*-desmethylvenlafaxine was significantly higher in the CYP2D6 EM group compared with the CYP2D6 HEM group ($p<0.02$), while the C/D ratios of venlafaxine did not differ significantly between the CYP2D6 EM and CYP2D6 HEM groups (Figure 3.3 a and 3.3 b). Also, the *O*-desmethylvenlafaxine/venlafaxine ratio was significantly higher in CYP2D6 EM compared with the CYP2D6 HEM group ($p<0.01$) (Figure 3.3 d). The C/D ratio of active moiety (venlafaxine plus *O*-desmethylvenlafaxine) did not differ significantly between the CYP2D6 EM and CYP2D6 HEM group (Figure 3.3 c).

The N-desmethylvenlafaxine C/D ratio was significantly higher (9 fold higher) between in the CYP2D6 HEM group compared to the CYP2D6 EM group ($p<0.01$). Nevertheless, the six patients in the HEM group were separated into two clusters (Figure 3.4 a). There were no significant differences in *N*-desmethylvenlafaxine/venlafaxine ratio between the two groups (Figure 3.4 b).

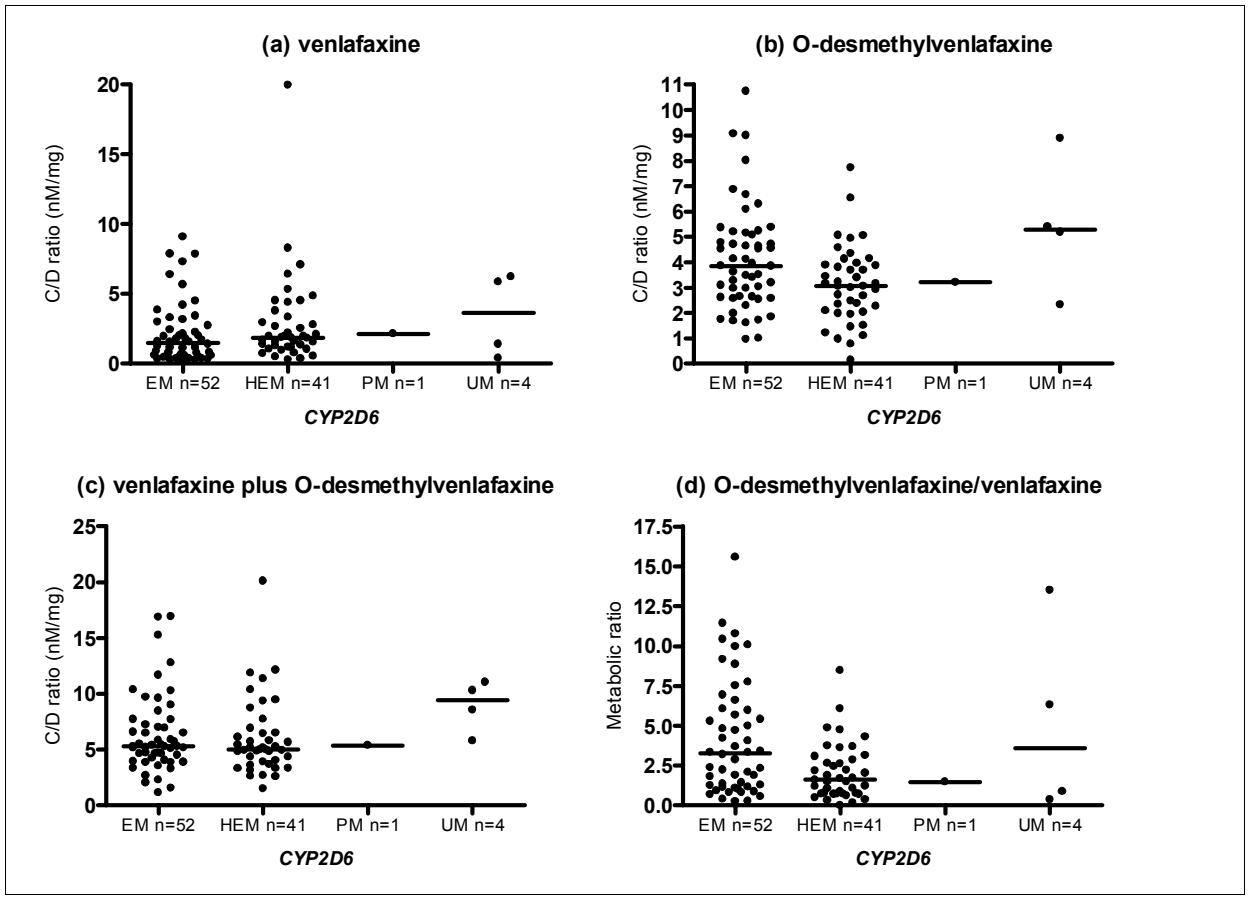

Figure 3.3 Relationship between the CYP2D6 genotype and C/D ratio of (a) venlafaxine, (b) *O*-desmethylvenlafaxine, (c) venlafaxine plus *O*-desmethylvenlafaxine and (d) metabolic ratio (*O*-desmethylvenlafaxine/venlafaxine).

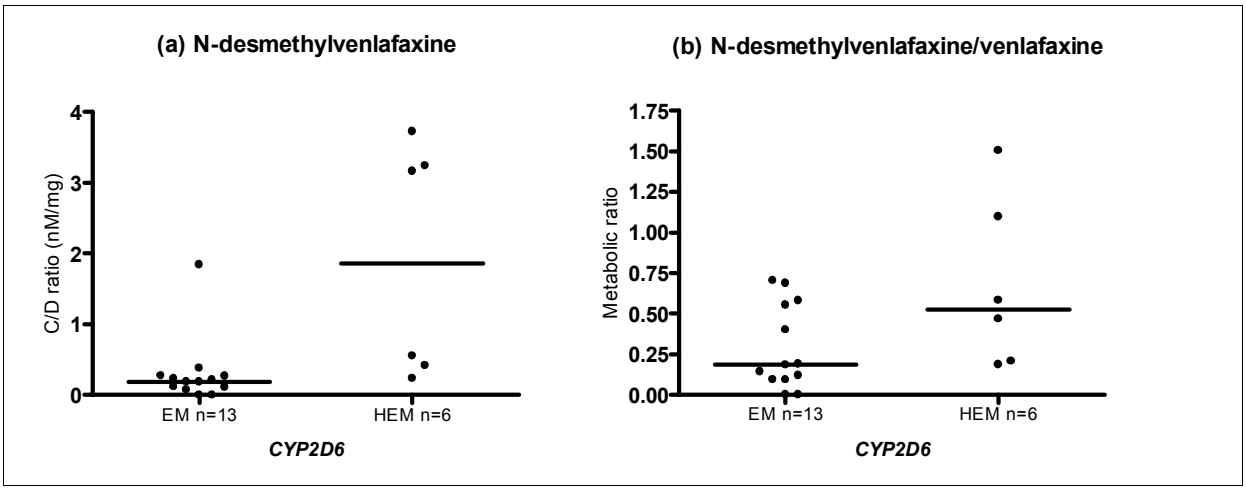

Figure 3.4 Relationship between the CYP2D6 genotype and (a) C/D ratio *N*-desmethylvenlafaxine and (b) metabolic ratio (*N*-desmethylvenlafaxine/venlafaxine).

A total number of 60 patients carried two functional CYP2C9 alleles and 25 patients carried one functional and one impaired CYP2C9 allele. The genotypes in this latter group were *1/*2 (n=14), CYP2C9*1/*3 (n=15). Among the rest, two patient carried two impaired CYP2C9 alleles, *2/*3. A total number of 57 patients carried two functional CYP2C19 alleles and 30 patients carried one functional and one defect CYP2C19 allele. The genotype in this latter group were *1/*2 (n=31). For both CYP2C9 and CYP2C19 there were 11 patients that were not genotyped. Effect of mutations in CYP2C9 and CYP2C19 was investigated in patients being either CYP2D6 EM or CYP2D6 HEM. There was a significant difference ($p<0.05$) in C/D ratio of active moiety (venlafaxine plus *O*-desmethylvenlafaxine), where patients being a combination of CYP2C9 HEM and CYP2D6 EM (n=17, median=5.6) had 21% higher median than patients being CYP2C9 EM and CYP2D6 EM (n=30, median=4.6). Despite the observed difference, patients with different mutations in CYP2C9 were included in the study because there was about the same frequency of CYP2C9 mutations in the CYP2D6 EM and HEM groups. Variation in CYP2C9 genotype therefore probably does not affect the results of the CYP2D6 analysis.

Of the 98 patients included in the study, six persons were receiving venlafaxine immediate release (IR) tablets, two of the patients were CYP2D6 EM and four of the patients were CYP2D6 HEM. The rest of the patients were receiving extended release (XR) tablets. Data of patients on IR tablets were plotted in the same plots as data of patients on XR tablets. The data of IR tablets were evenly distributed among the data of XR tablets and did not differ from the data for the IR tablets, therefore all data were analyzed together.

4. DISCUSSION

4.1 Risperidone tablets and risperidone long-acting injection

This is the first analysis to investigate the influence of CYP2D6 genotype on serum concentration for risperidone long acting injection. A significant influence of CYP2D6 genotype for serum concentration of risperidone tablets has been previously shown (Scordo et al, 1999). An analysis comparing oral and long-acting risperidone showed that patients receiving oral risperidone had about four-fold higher plasma metabolite/drug ratio compared to patients receiving the new depot formulation (Nesvåg et al, 2006). The lower level of 9-hydroxyrisperidone in the depot group may be a result of reduced first-pass metabolism (Nesvåg et al, 2006). Because the influence of CYP3A4 and CYP2D6 probably are different for the two formulations, it was of interest to see whether CYP2D6 polymorphism has different impact on the two formulations.

In patients medicated with risperidone tablets, the parent compound risperidone was over eight times higher in CYP2D6 HEM compared to CYP2D6 EM. This was not observed in a previous study where CYP2D6 PM had significantly higher risperidone than the other genotypes, but there were no significant differences for the CYP2D6 HEM patients (Scordo et al, 2006). This difference might be a result of a higher number of patients in our analysis (n=23 versus n=15). In spite of the differences in serum concentrations of risperidone between CYP2D6 EM and HEM, there are no significant differences between the two genotypes in the active moiety (sum of risperidone and 9-hydroxyrisperidone). This is probably due to increased elimination of risperidone through CYP3A4, the alternative metabolism pathway of risperidone (Figure 1.1). In contrast to the study by Scordo and co-workers where significantly higher risperidone/9-hydroxy risperidone ratios were observed in CYP2D6 HEM and PM versus CYP2D6 EM, no difference in this ratio was observed in the present analysis. This is probably due to a large variability in our data (Figure 3.1 d). Probable causes of this variability include possible partial non-compliant patients, drug intake that we are unaware of, for instance CYP3A4 inducers carbamazepine or St. Johns wort. In addition, the patients might be misclassified as HEM as only 30% of UM can be detected by genotyping, or they might have rare mutations that was not detected in the genotyping. The terminal half-life of risperidone and 9-hydroxyrisperidone are about 6-7 hours and about 24 hours, respectively.

The serum samples have been withdrawn between 12 and 24 hours and the samples withdrawn closer to 24 hours therefore have higher 9-hydroxyrisperidone/risperidone ratio than the samples withdrawn closer to 12 hours.

For risperidone long-acting injection, the 9-hydroxyrisperidone/risperidone ratio was over twice as high for CYP2D6 EM patients as for HEM patients. In contrast to risperidone tablets there was no difference in serum concentration of risperidone for risperidone long-acting injection. However, the differences between risperidone long-acting injection and risperidone tablets might be due to a difference in the influence of CYP3A4. As there is more CYP3A4 than CYP2D6 in the intestine (Lin, Lu, 2001), the difference in administration route may result in different relative significance of the two enzymes for the two formulations.

Studies indicate that P-glycoprotein (P-gp) in the blood-brain-barrier (BBB) reduces the brain concentration of risperidone and 9-hydroxyrisperidone by limiting their CNS-access (Nakagami et al, 2005, Wang et al, 2004). 9-hydroxyrisperidone has been shown to be more effectively transported by P-gp than risperidone through the BBB, as the ratio of brain to plasma of 9-hydroxyrisperidone in P-gp knockout mouse was 2.4 fold higher than that of risperidone (Wang et al, 2004, Doran et al, 2005). The distribution of 9-hydroxyrisperidone to the different brain-regions, including frontal cortex and striatum, has also been shown to be more limited than for risperidone itself (van Beijsterveldt et al, 1994). Patients with higher serum levels of risperidone, such as CYP2D6 HEM patients, will therefore probably obtain higher active brain concentrations compared to CYP2D6 EM patients. This possible difference in concentration at site of action will probably also cause a difference in effect.

The CYP2D6 EM patients in our analysis received about 15% higher doses of risperidone long-acting injection than the CYP2D6 HEM patients. This could be due to a better toleration of higher doses in CYP2D6 EM compared to HEM. This is in accordance with an elevated prevalence of adverse-drug reactions and discontinuation in CYP2D6 PM patients (de Leon et al, 2005). This further supports our hypothesis that a change of risperidone makes a difference for effect/side-effect, even though sum of risperidone plus 9-hydroxyrisperidone and/or the ratio not have changed.

4.2 Venlafaxine

In the present analysis, the level of *O*-desmethylvenlafaxine was about 25% higher in CYP2D6 EM than in HEM. This difference was not observed by Shams and co-workers, probably due to a low number of patients (n=5) compared to our analysis (n=41). The level of venlafaxine did not differ significantly between CYP2D6 EM and HEM. The lack of increase in venlafaxine could be caused by increased formation of *N*-desmethylvenlafaxine through CYP3A4 when CYP2D6 metabolism is impaired (Figure 1.1). This is supported by significantly higher serum concentrations in *N*-desmethylvenlafaxine in CYP2D6 HEM versus EM patients shown in the present analysis (Figure 4.1). Also, there seems to be corresponding lower *O*-desmethylvenlafaxine levels in patients with high *N*-desmethylvenlafaxine and vice versa (Figure 4.1). It would have been useful with more patients in this analysis, but analysis of *N*-desmethylvenlafaxine has only been measured at Department of Psychopharmacology, Diakonhjemmet hospital since March 2006.

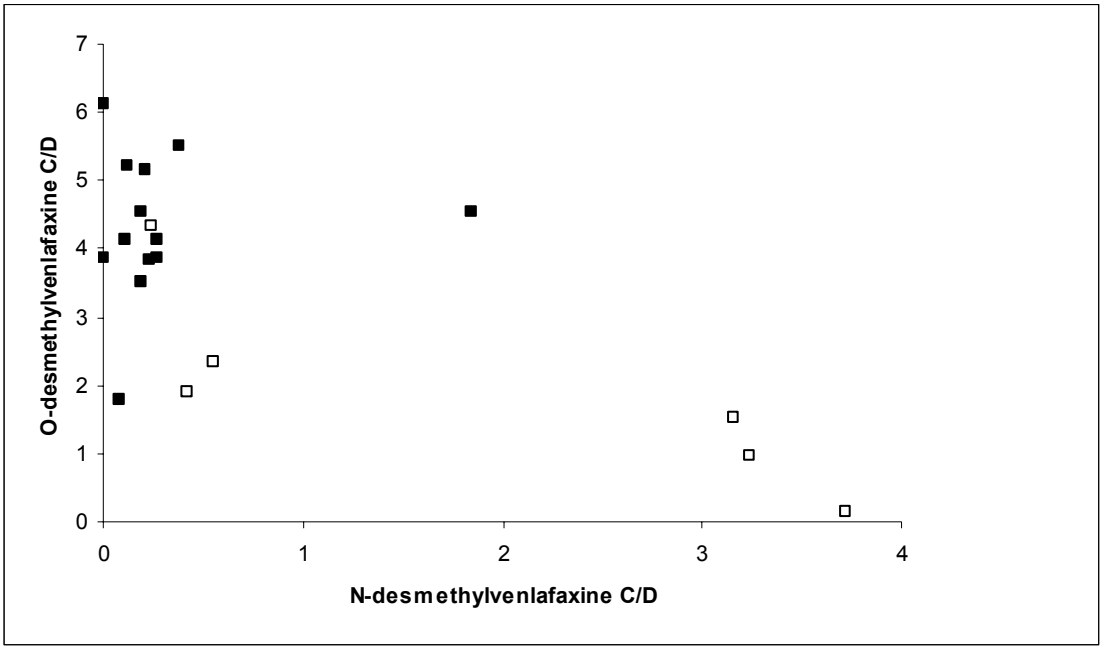

Figure 4.1 -dose ratios of *O*-desmethylvenlafaxine and *N*-desmethylvenlafaxine
In (■) CYP2D6 extensive metabolizers (n=13) and (□) CYP2D6 heterozygous metabolizers (n=6).

In spite of the differences in C/D ratio of *O*-desmethylvenlafaxine between CYP2D6 EM and HEM, there were no significant differences between the genotypes in the active moiety (venlafaxine plus *O*-desmethylvenlafaxine). The ratio of *O*-desmethylvenlafaxine/venlafaxine was, however twice as high in CYP2D6 EM patients as in CYP2D6 HEM patients. *O*-desmethylvenlafaxine displays similar serotonin reuptake inhibition activity and two-fold higher noradrenalin reuptake inhibition than venlafaxine (Muth et al, 1986) and a change in the ratio of metabolites might therefore also result in a change in effect. It has been observed that side effects of venlafaxine like nausea, vomiting and diarrhea are increased in CYP2D6 PM compared to EM (Shams et al, 2006). Correspondingly, the risk of side effects increases with decreasing ratio of *O*-desmethylvenlafaxine/venlafaxine (Shams et al, 2006). The authors blamed this effect on the increased levels of parent compound venlafaxine (Shams et al, 2006). However both venlafaxine and *N*-desmethylvenlafaxine are increased and both substances increase in the same patient category that has the observed side-effects. The side-effects might therefore just as well be due to the increased level of *N*-desmethylvenlafaxine that also appears in patients with reduced CYP2D6 capacity. The percent increase in serum C/D ratio for CYP2D6 HEM patients compared to CYP2D6 EM patients is higher for *N*-desmethylvenlafaxine (850% higher levels of N-desmethylvenlafaxine in CYP2D6 HEM compared to CYP2D6 EM) than for venlafaxine (26% higher levels of venlafaxine in CYP2D6 HEM than CYP2D6 EM). A further complicating factor is that venlafaxine is a chiral drug, and that CYP2D6 displays marked stereoselectivity towards the *(R)*-enantiomer. This could have clinical consequences and thus underlie the observed differences in the occurrence of side effects in CYP2D6 PM and EM (Eap et al, 2003). This issue needs further investigation, and from current data available it is not possible to point to which of the compounds that causes side-effects.

It was expected that a possible influence of CYP2C9 and CYP2C19 genotypes on the serum concentration of venlafaxine would be best uncovered by investigating the group of patients that were CYP2D6 HEM because the main metabolism pathway is reduced in these patients. Surprisingly, there was a 21% higher active moiety (sum of venlafaxine and *O*-desmethylvenlafaxine) in the CYP2D6 EM patient group with a higher median of CYP2C9 EM than the median of 2C9 HEM. The observed significant influence of mutations in CYP2C9 in CYP2D6 EM patients and not in CYP2D6 HEM patients can not be explained by current available data/literature, and more patients are needed to investigate the possible influence of genotype for serum concentration on these enzymes separately.

4.3 Clinical implications

In common for risperidone and venlafaxine is that the active moiety not is changed in CYP2D6 HEM versus EM, but there are changes in the ratios of parent drug and metabolite. Today, the plasma concentration sum of drug and active metabolite is used in TDM practice for both these drugs (Baumann et al, 2004). Wanted and unwanted drug effects depend on the concentration on the site of action. The brain is not accessible to drug monitoring, but plasma concentrations are thought to reflect brain concentrations. However, animal studies have shown variable brain distribution of parent compounds and active metabolites for both risperidone and venlafaxine.

Serum/plasma average of 9-hydroxyrisperidone is higher than the concentrations of risperidone itself, 90-95% of the drug is present as 9-hydroxyrisperidone in plasma (Olesen et al, 1998, Riedel et al, 2005). Animal studies have shown at least five-fold more extensive distribution of risperidone and at least two-fold more extensive distribution of venlafaxine in brain compared to their active metabolites (Doran et al, 2005, Uhr et al, 2003, Wang et al, 2004). The pharmacological activity of 9-hydroxyrisperidone is approximately 70% comparable to risperidone in respect to activity (Van Bejsterveld et al, 1994). *O*-desmethylvenlafaxine displays similar serotonin reuptake inhibition activity and two-fold higher noradrenalin reuptake inhibition than venlafaxine (Muth et al, 1986). This indicates that 9-hydroxyrisperidone and *O*-desmethylvenlafaxine contributes to the *in vivo* activity of the drugs, but to a smaller extent than would be predicted from plasma levels. Whether these differences in distribution is present in humans remains to be investigated, but if so, variable plasma metabolite/parent drug ratios caused by genetic polymorphism of drug metabolizing enzymes, metabolic drug-drug interactions or different administration routes could reflect different active concentrations in the brain, despite similar total concentrations of drug and metabolite in plasma. A change in metabolite ratio for risperidone and venlafaxine might therefore contribute to variation in effect/side-effect of the drugs.

4.4 Strengths and weaknesses in the studies

This analysis, with data from a naturalistic clinical setting, includes a relatively high number of patients. Age and sex are equally distributed between the CYP2D6 EM and HEM, and differences in serum concentrations are therefore most likely due to differences in genotypes.

In this analysis the C/D ratio has been used in order to compare serum concentrations for patients on different drug dosages. This dose-corrected serum concentration is a presumption of that the drugs follows linear kinetics, which is not necessarily correct. For risperidone long-acting injection, there was a significant difference in dosage between the CYP2D6 EM versus HEM group, which is a possible source of error.

These analyses are based on information given from the prescribing clinician and there is no direct contact with the patients. The patients might therefore be medicated with drugs that we are unaware of, for instance CYP2D6 inhibitors or CYP3A4 inducers or inhibitors.

Patients might be classified into wrong genotype groups because of undetected mutations. *CYP2D6*9, *10* and **17* are not genotyped in this analysis. Studies have indicated a *CYP2D6*9* allele frequency of 1-2% in Caucasians and *CYP2D6*10* allele frequency of <2% in Caucasians (Bradford, 2002). In addition, *CYP2D6*10* mutated alleles are more common in Asians immigrants, which not are excluded from this study, with an allele frequency of about 41% (Bradford, 2002). The *CYP2D6*17* is very rare in Caucasians (Bradford, 2002) but is a mutation common among Africans and African-Americans with a frequency of about 24% and might appear in African and African-American immigrants analyzed in this study. Another mutation not genotyped in this analysis is *CYP2C19*17*. This mutation increases metabolism of drugs metabolized by CYP2C19 and have a frequency of approximately 18% in Caucasians (Sim et al, 2005).

5. Conclusion

Common for risperidone and venlafaxine is that even though the sum of the active moiety not is changed, the ratio of metabolite/ parent drug is. It is the sum of the active moiety that is determined in TDM, and patients are therefore treated equally because the sum will be the same independent of genotype. This is a problem if there is a difference in effect/adverse drug reactions mediated by parent drug and metabolite. A changed metabolite ratio might therefore be considered as a possible cause of unexpected clinical response. More knowledge of possible differences for the metabolite versus parent drug in for instance receptor affinity and/or distribution is necessary to optimize the treatment with these drugs in relation to the patient genotype.

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